ON THE PRESENCE OF A FEEDING HORMONE IN THE NEMATOCYST OF HYDRA PIRARDI

ALLISON L. BURNETT, RICHARD DAVIDSON AND PETER WIERNIK

Developmental Biology Center, Department of Biology, Western Reserve University, Cleveland, Ohio, and University of Virginia Medical School, Charlottesville, Virginia

Loomis (1955) reported that the feeding response in hydra is specifically stimulated by reduced glutathione oozing from the tissues of the prey animal after the penetration of the animal by hydra nematocysts. Recently, Forrest (1962) reported a lack of dependence of the feeding reaction in hydra on GSH. She cites numerous past publications which do not confirm the notion that hydra must feed on living animals containing GSH in their body cavities. In addition, she lists other compounds which mimic the effect of GSH in that they produce the typical tentacle movements and mouth-opening characteristic of the feeding response.

The present paper demonstrates: (a) that all scientists have not uncritically accepted "the GSH story," (b) that hydra is *not* necessarily stimulated to feed by substances issuing from the prey animal after nematocyst penetration, (c) that it is not known at the present whether GSH is or is not the specific stimulus to feeding in nature.

I. Substances Other Than Reduced Glutathione Which Will Stimulate the Feeding Reflex in Hydra

No attempt will be made here to review the papers which claim that substances other than GSH are capable of stimulating a feeding response in hydra. (See review of Forrest, 1962.) Suffice it to say that in addition to GSH, lactic acid, ascorbic acid, acetic acid, sodium acetate, ophthalmic acid, norophthalmic acid, papain, ficin, trypsin, quinine hydrochloride, plus other compounds, have been reported to elicit the response. In addition, we have observed the response after treating hydra with hyaluronidase, reduced methylene blue, dilute NaOH, and HCl.

From these observations it appears unlikely that GSH is the sole stimulus for a specific feeding reflex even in nature. Why do these diverse substances activate the feeding response?

Experiments conducted during the past two years have led us to conclude that in nature GSH may or may not be the sole stimulus of the hydra feeding response and that substances other than GSH (excluding GSH analogues) may cause a feeding response through a mechanism different from that activated by GSH.

First, let us consider the activation of the feeding reflex by GSH. When prey strikes the tentacles of the hydra, penetrant nematocysts puncture its tissues. Body fluids then ooze from the wound produced by the nematocyst thread. Loomis (1955) suggested that these body fluids contain GSH and that this compound stimulates the feeding response in hydra. Recently, Lenhoff (1961) demonstrated that hydra immediately begin the feeding response when they are placed in a 10^{-5} *M*

GSH solution. The mouths of these animals remain open for as long as 40 minutes. When washed in fresh culture water, they fail to respond maximally to GSH for 24 hours.

A slight modification of this experiment produced some instructive results. Forty hydra (*Hydra pseudoligactis*), starved for 48 hours, were placed in a Petri dish containing a 10^{-5} *M* GSH solution. The animals exhibited normal feeding response. After one hour all of the hydra had closed their mouths. To demonstrate that GSH was still present in the medium, five starving hydra were introduced into the same Petri dish. These animals immediately opened their mouths and began the feeding response. *Artemia* were then introduced into the Petri dish. Every one of the 40 animals which no longer responded to the GSH stimulus killed several *Artemia,* began a normal feeding response and ingested the *Artemia* normally. These results suggest that the contact of living *Artemia* with the tentacles of the hydra either reactivates the original GSH receptor site or stimulates another mechanism which is not related to GSH. In any case it has been demonstrated that hydra which no longer respond to a GSH stimulus *will* execute a feeding response when offered living *Artemia.* This observation casts doubt on the notion that GSH oozing from the nematocyst wound is the sole stimulus to the feeding reaction in hydra.

An explanation of substances other than GSH which will induce the feeding reflex has suggested that these substances operate through a mechanism which may be related only indirectly to the GSH mechanism. The substances tested in the present experiments were lyophilized crystalline trypsin (Armour Labs, Chicago, buffered to pH 6.3, employed in a concentration of 0.1 mg./ml.), hyaluronidase (Nutritional Biochemicals, Cleveland, buffered at pH 6.2, concentration 1 mg./ml.), lactic acid (10^{-4} *M,* pH 5). *Hydra pseudoligactis* which had been starved for 48 hours were introduced by means of microforceps into 1-ml. test solutions and observed with a binocular dissecting microscope at 20 × or with a compound light microscope at 100 ×.

Five hydra were placed in the test solution in a depression slide and observed at 100 ×. Trypsin, hyaluronidase, and lactic acid stimulated stenotele discharge from the tentacles. These chemicals appeared to lower the threshold necessary to stimulate the discharge of this particular nematocyst type. For instance, a hydra in a trypsin solution discharged as many as three dozen stenoteles from a limited area of a single tentacle when the tentacle contacted debris. Moreover, when one tentacle of the hydra contacted another tentacle there was a nematocyst discharge between the tentacles, and the hydra discharged stenoteles into its own tissues. Often the tentacles stuck to one another because of the nematocyst discharge from an adjacent tentacle, and a muscular exertion was necessary before hydra could separate its tentacles. Another reaction was also evident. If the hydra curled a single tentacle so that the extremity of the tentacle contacted more proximal regions of the same tentacle, there was a prompt discharge of stenoteles. Finally, nematocyst discharge was seen in tentacles which were not stimulated by either the glass slide or another tentacle.

Continued observations revealed that the hydra never opened their mouths nor began the feeding reaction until several stenoteles were discharged. It appeared that perhaps nematocysts piercing the tissues of the hydra itself were producing

wounds through which GSH in the epithelio-muscular cells of the hydra could pass into the test solution and stimulate the feeding response. More will be said concerning this point later. It must be stressed that animals which were placed in a depression slide containing normal culture medium (Versene-bicarbonate solution—see Loomis and Lenhoff, 1956) did not discharge excess stenoteles either when the tentacles contacted each other or when they contacted the bottom of the slide.

In order to quantitate the increased stenotele discharge in test solutions, the following experiment was conducted. Five experimental animals, starved for 24 hours, were placed in 1 ml. of each test solution and examined. After these animals opened their mouths and were passing their tentacles through the mouth opening into the enteron, they were removed from the test solution with microforceps and placed on a clean glass slide. Five drops of a 10% alcohol solution (which paralyzes the animals and permits the investigator to count discharged stenoteles) were placed on the hydra, and a very light squash preparation was made by placing a coverslip over the animals. This squash preparation was then examined at

TABLE I*

Culture medium	% of animals which opened mouths and carried on normal feeding reflex	No. of discharged stenoteles. Average for 5 animals
1. Normal	0%	17
2. Trypsin	100%	64
3. Hyaluronidase	80%**	36
4. Lactic acid	100%	50
5. GSH	100%	18

* It must be remembered that the number of stenoteles recorded in these experiments was probably less than the number which had been discharged in test solution because several discharged stenoteles were perhaps lost when the hydra was transferred from the test solution to the glass slide.

** One hydra did not respond to the hyaluronidase solution. Subsequent examination revealed that this animal released only three stenoteles. This figure significantly lowers the average number of stenoteles which were discharged for the group of 5.

440 × and the number of discharged stenoteles was recorded. To ensure that excess stenoteles were not discharged in response to the 10% alcohol solution or the pressure of the coverslip, a control group of hydra was taken from the normal culture medium, placed in the alcohol solution and examined. The results of this experiment are seen in Table I.

It will be seen that the two enzymes and lactic acid stimulated a stenotele discharge which was at least double that of animals in normal culture medium or those in a GSH solution. Animals which were placed in a GSH solution in a depression slide and viewed during the feeding reflex at 100 × did not release stenoteles; limited stenotele discharge occurred only when these animals were crushed under a coverslip in an alcohol solution. Animals placed in lactic acid and hyaluronidase solutions did not open their mouths within 5–15 seconds as did the hydra placed in a GSH solution. As long as 30 seconds–two minutes elapsed before the feeding reaction occurred. Animals placed in a trypsin solution

opened their mouths after 25 seconds to one minute. The response here is slower than that of animals in a GSH solution but faster than that of animals in hyaluronidase or lactic acid. These results may indicate that trypsin stimulates a much greater stenotele discharge than either hyaluronidase or lactic acid.

These results suggest that substances other than GSH or its analogues might stimulate the feeding reflex, not through an activation of a GSH receptor in hydra, but by stimulating stenotele discharge which ultimately results in a release of GSH from the tissues of the hydra itself. To test this hypothesis the following experiment was conducted. It is well known that strong acids or alkalis stimulate nematocyst discharge. Balke and Steiner (1959) were able to stimulate a feeding reaction in hydra with acetic acid. In our laboratory we commonly stimulated nematocyst discharge with dilute NaOH. Thus, if NaOH elicited a feeding reaction in hydra, then more support would be given to the hypothesis that stenoteles contain the feeding hormone.

Fifteen hydra starved for 48 hours were placed into a Syracuse watchglass containing 10 cc. 10^{-5} *M* NaOH. Within one minute three of these animals had opened their mouths and were carrying out a perfectly normal feeding reflex. The tentacle movements were well-coordinated, the mouth opened in a manner similar to that observed when hydra were placed in a GSH solution. This is not the "gaping" that Lenhoff (1961) has reported when hydra are placed in a solution of noxious compounds. The remaining 12 animals, although exhibiting tentacle movements characteristic of the feeding response, had not opened their mouths after one minute. At this time the tip of a thin dissecting needle was dipped into a solution of 10^{-2} *M* NaOH. The needle was then introduced into the culture medium and placed between the tentacles of animals which had not yet opened their mouths. The tentacles of these forms immediately began a more active feeding response and within a few seconds the mouths opened. By successively dipping the needle into concentrated NaOH solution and stimulating hydra which had not opened their mouths, we were able to stimulate mouth-opening in 12 of our 15 test animals. These animals were then placed in normal culture medium and were all in a healthy condition the following day.

II. Permeability Properties of the Nematocyst Capsule

These results could mean that substances other than GSH evoke a feeding response by stimulating a nematocyst discharge which causes the hydra to penetrate its own tissues with stenoteles, thereby introducing GSH into the surrounding medium. Further observations on the permeability of the nematocyst capsule forbid this conclusion.

Methylene blue readily enters and remains for several days within the stenoteles of the intact hydra. However, isolated, undischarged nematocysts when stained with methylene blue readily lose the stain when transferred to a fresh culture medium. Also, fully developed nematocysts in isolated cnidoblast cells similarly lose the dye to the surrounding medium when removed from the methylene blue solution. In short, only the intact hydra or portions of intact animals retain methylene blue within the nematocyst capsule.

These observations suggested that once the nematocyst thread had everted and projected beyond the cnidoblast cell, the contents of the nematocyst capsule which

had not been discharged through the end of the thread might leak from the butt of the exposed capsule. The contents of the capsule of the stenotele when liberated into the surrounding medium might be capable of eliciting a feeding response. In this case the "environmental hormone" postulated by Loomis would be located in the tissues of the hydra itself.

III. Stimulation of the Feeding Response Without Chemical or Mechanical Stimulation

In order to test this hypothesis, it was necessary to elicit a feeding response in hydra without relying upon chemicals applied to the external medium. This was accomplished by employing a method of nematocyst discharge recorded by Kline (1961) who employed electric shock to stimulate a massive nematocyst discharge.

In a typical experiment, four *Hydra pirardi,* starved for 24 hours, were placed in a drop of distilled water on a slide. A 72-volt shock from 12 6-volt batteries was applied for a period of one second. These hydra immediately were removed with microforceps and two normal individuals were placed in the drop of water. These animals promptly opened their mouths and began a normal feeding response. After a few minutes two more animals were introduced and they too began the feeding response. This maneuver was continued until eight animals were introduced into the medium. The feeding responses lasted for as long as 30 minutes. Several of the animals began to spread their mouths over the bottom of the slide in an attempt to devour it.

Similar results were obtained when excised tentacles were shocked in a drop of water. These tentacles were well separated so that they could not come into contact with one another and were observed during the shock to insure that they did not curl and come into contact with themselves. After one second of electrical stimulation the tentacles were immediately removed and ten hydra which were subsequently introduced into the solution began the feeding response. These results suggest that a substance within the nematocyst capsule itself stimulates a feeding response in hydra.

One question comes to mind. Burnett (unpublished observations) has observed that hydra may discharge only a few stenoteles to subdue a prey animal: is this discharge sufficient to stimulate a feeding reaction? The answer is "yes," provided the proper mechanical stimulation is present. We have confirmed the observations of Lenhoff (1961) that hydra which remain for long periods of time in a GSH solution eventually close their mouths. However, it has been demonstrated that if these same animals are stimulated by teasing their tentacles with microforceps or transferred to a slide with a pipette they will open their mouths and begin a feeding response.[1] This explains why hydra which have habituated to a GSH solution will readily accept and devour *Artemia.*

We have demonstrated that if a 48-hour starved hydra is picked up with a pipette and dropped upon a slide, as many as 20 stenoteles are discharged. These animals do not open their mouths but will wave their tentacles in a manner which resembles that of the feeding response. However, if the tentacles of these indi-

[1] Recent experiments with *H. littoralis,* obtained from Lenhoff, have demonstrated that unlike *H. pirardi, H. littoralis* will not respond after it is habituated to GSH.

viduals are stimulated by rubbing microforceps along their length, mouth-opening invariably follows. The mouth will remain open for varying periods of time, depending upon the number of nematocysts discharged. When hydra captures a prey animal, it invariably brings the animal into contact with the mouth. Once the mouth opens, the prey is invariably ingested.

IV. Specificity of Glutathione in the Feeding Reflex

All of our experiments indicate that any substance which stimulates nematocyst discharge will stimulate the feeding reflex. Acids and alkalies are traditionally employed in classrooms to stimulate nematocyst discharge. However, most of these materials are noxious and if not used in extremely dilute solutions will cause the death of the animal. From the foregoing experiments it appears that lactic acid, ascorbic acid, quinine hydrochloride, hyaluronidase, trypsin, acetic acid, sodium hydroxide, electric shock, glutamic acid, sodium acetate, etc., stimulate nematocyst discharge and it is this discharge which evokes the feeding reflex, not the chemicals themselves. Other substances which stimulate the feeding response, such as beef broth, egg white, etc., may also stimulate a nematocyst discharge. At the present time, it is not known whether the factor within the nematocyst capsule is GSH.

V. Evolutionary Speculations

Loomis (1955) stated that hydra feed upon only those animals which liberate sufficient quantities of GSH after penetration by the hydra nematocyst. Loomis suggests that GSH would be found in this high concentration only in those forms which possessed a fluid-filled body cavity (*e.g.*, annelids). Upon puncture of these forms by nematocysts, fluids containing GSH would apparently ooze from the wound. For this reason hydra feeds, according to Loomis, only on forms such as annelids and arthropods whose coelom and haemocoele, respectively, would represent a fluid-filled body cavity.

First we agree with Forrest (1962) that hydra are not forced to feed only upon members of certain select phyla. We have found, for example, that a brown hydra (*Hydra oligactis*) may be dried in a desiccator for 48 hours, and the tiny dried piece of tissue will be ingested if dropped into the middle of the circlet of tentacles surrounding the mouth of a hungry animal of the same species. We also have observed that the brown hydra (*Hydra pirardi*) will kill and ingest a species of European green hydra (*Hydra viridis*). It is impossible to culture these two species in the same dish for this reason.

It is our contention that the hydra is not restricted to diet by the presence of a body cavity in the host animal, but depends on whether or not the prey organism elicits a stenotele discharge when it contacts the tentacles of the hydra. We would be very surprised indeed if substances like egg white, dried hydra, protozoans, and even mud, all of which have been claimed to elicit the feeding response, did not stimulate a discharge of stenoteles upon contact with the tentacles. Mechanical stimulation, coupled with stenotele discharge, will elicit a feeding response if only a few stenoteles have been discharged.

This observation explains perhaps how primitive coelenterates were able to feed with nematocysts. If one postulates that a body cavity must exist in the prey

organism before the hydra can feed, then one may ask, what was the source of food materials for coelenterates before the higher coelomate phyla evolved? If one postulates that the "hormone" which stimulates the feeding response evolved within the capsule of the stenotele, then any animal, irrespective of body cavity, which stimulated nematocyst discharge could presumably be ingested.

It is not surprising that Loomis presented evidence that hydra does not feed upon other hydra or upon flatworms. First, many flatworms do not stimulate nematocyst discharge when placed on the tentacles of hydra. For example, we have never been able to elicit a feeding response from *Hydra pseudoligactis* by offering this form *Dugesia* sp. Moreover, most hydra will not discharge nematocysts against other species of hydra. In our experience only the green hydra will stimulate nematocyst discharge from *Hydra pirardi*. It is not inconceivable, however, that similar relations might exist between certain species of brown hydra.

At the present time, the only coelenterate which we have studied in terms of the feeding response is the common hydra. We urge that investigators study the feeding mechanism in marine forms in light of nematocyst discharge. Care must be taken to insure that the type of nematocyst discharged is the one employed by the animal to pierce the prey animal, not specialized nematocysts employed in locomotion, defense, etc. Once nematocyst discharge has taken place, then the tentacles should be immediately stimulated mechanically.

VI. Hydra Without Nematocysts

From the foregoing observations it might be concluded that a hydra which lacks nematocysts is incapable of exhibiting a feeding response. This is not the case. If the hypostome and tentacles are removed from a hydra and then the tentacles trimmed off at the junction with the hypostome, the isolated hypostome will not open its mouth when stimulated with lactic acid or hyaluronidase, both of which stimulate nematocyst discharge. However, these isolated hypostomes will respond for a short period of time to GSH which does not specifically stimulate nematocyst discharge.

This gives us strong reason to believe that GSH does not act directly through nematocysts in eliciting a feeding response. In fact, it is conceivable that GSH is actually present in the capsule of the stenotele and is still the compound ultimately responsible for the feeding response. It would be a precarious situation indeed for the hydra to depend upon the presence of a single molecule in the prey animal in order to feed. However, if this molecule were built into the animal's own system, it would be of definite selective advantage to the animal. On the other hand, it is possible that compounds other than GSH which are capable of eliciting a feeding response are located in the nematocyst capsule.

Summary

1. It is not necessary to postulate an environmental hormone in order to explain the feeding response in hydra. Since nematocysts which are removed from the tissues of the hydra do not retain small molecules (methylene blue) which they readily accept and bind in the intact animal, it is suggested that during nematocyst discharge, in addition to the introduction into the prey of a toxic material, a certain

substance or substances leak from the butt of the nematocyst capsule and diffuse into the surrounding medium. This substance(s) is capable of stimulating mouth-opening when it comes into contact with the hypostome. Dilute concentrations of the substance which will not elicit a feeding response are found to do so if the tentacles are stimulated mechanically. This explains how a few stenoteles, when discharged into an actively wriggling prey animal, are sufficient to stimulate mouth-opening.

2. Although there is evidence that GSH is not the specific evocator of the feeding response in hydra in nature, more thorough studies must be conducted. All of the materials which stimulate a feeding response and lack GSH may be shown to stimulate nematocyst discharge, and as we have suggested, the nematocyst may contain GSH.

3. It is suggested that for *H. pseudoligactis* and *H. pirardi,* at least, the term "environmental hormone" be dropped. If this primitive hormone does exist in the capsule of the stenotele the term retro-hormone might be more appropriate.

LITERATURE CITED

Balke, E., and G. Steiner, 1959. Über die chemische Nahrungswahl von *Pelmatohydra oligactis* Pallas. *Naturwiss.,* **46**: 22.

Forrest, H., 1962. Lack of dependence of the feeding reaction in Hydra on reduced glutathione. *Biol. Bull.,* **122**: 343–361.

Kline, E., 1961. Chemistry of the nematocyst capsule and toxin of *Hydra littoralis. In:* The Biology of Hydra; edited by H. M. Lenhoff and W. F. Loomis. The University of Miami Press, pp. 153–168.

Lenhoff, H. M., 1961. Activation of the feeding reflex in *Hydra littoralis. In:* The Biology of Hydra; edited by H. M. Lenhoff and W. F. Loomis. The University of Miami Press, pp. 202–232.

Loomis, W. F. 1955. Glutathione control of the specific feeding reaction in Hydra. *Ann. N. Y. Acad. Sci.,* **62**: 209–228.

Loomis, W. F., and H. M. Lenhoff, 1956. Growth and sexual differentiation of Hydra in mass culture. *J. Exp. Zool.,* **132**: 555–574.